

#### **Research Article**

# A new species and a replacement name in *Cynanchum* (Apocynaceae, Asclepiadeae) from China

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#### Abstract

*Cynanchum pingtaoi* S.Jin Zeng, G.D.Tang & Miao Liao, **sp. nov.** (Apocynaceae) from Yunnan Province, China, is described and illustrated based on morphological and molecular evidence. Its deeply cordate to reniform leaves and campanulate, large flowers show that it is a member of former *Raphistemma* Wall., which has been included in *Cynanchum* L.. It is different from all former *Raphistemma* species by the broadly ovate corolla lobes, purple-red corolla and connivent corona tip slightly exceeding the corolla throat. Meanwhile, *Cynanchum longhushanense* G.D.Tang & Miao Liao, **nom. nov.** is proposed as replacement name for *Raphistemma brevipedunculatum* Y.Wan, which was considered a synonym of *Cynanchum hooperianum* (Blume) Liede & Khanum but is here reinstated as a distinct species because of significant morphological differences.

**Key words:** Cynanchum hooperianum, Cynanchum longhushanense, Cynanchum pingtaoi, morphology, phylogeny, *Raphistemma*, taxonomy

# Introduction

*Cynanchum* L. is one of the largest genera of Asclepiadeae (Apocynaceae) and is mainly distributed in Africa and Asia, but it occurs as well in the New World (Khanum et al. 2016; Endress et al. 2018). It is considered a "dustbin genus" due to its complex morphological characteristics, but species generally possess a gynostegial, at least basally fused corona (Liede and Kunze 1993). The extent and classification of *Cynanchum* are controversial. Markgraf (1972) and Ali and Khatoon (1982) supported *Cynanchum* and *Vincetoxicum* Wolf as separate. However, other studies did not support this circumscription (Tsiang and Li 1977; Li et al. 1995). Woodson (1941) and Liede (1997) suggested *Cynanchum* should include more than twenty allied genera but not *Vincetoxicum*.



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Copyright: © Miao Liao et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Recent studies, based on molecular and morphological evidence, changed the *Cynanchum* circumscription, including more than ten genera (e.g., *Raphistemma* Wall.) (Khanum et al. 2016) and segregating *Vincetoxicum* (Qiu et al. 1989; Liede 1996, 2001; Rapini et al. 2007). A wide concept of the genus with about 250 spp. has been accepted (Khanum et al. 2016; Endress et al. 2018), and the newly circumscribed *Cynanchum* generally comprises species which possess a corolla 3-10 (-40) mm long, a highly variable gynostegial corona, usually with a ring of fused staminal and interstaminal parts, the staminal parts occasionally with an adaxial appendage, occasionally with additional free staminal lobes connate to ring (Endress et al. 2018).

Cynanchum is a large and complex genus, and the Chinese representatives of Cynanchum have not been completely revised since the "Flora of China" (Li et al. 1995). The number of species of Chinese Cynanchum recorded in databases and literature references differs considerably, e.g., "Flora of China" reports 57 species of Cynanchum in China (Li et al. 1995); Plants of the World Online (POWO, https://powo.science.kew.org/) cites 45 species (including C. heydei Hook.f. distributed in Xizang, China and C. lanhsuense T.Yamaz. which occurs only in Taiwan, China); Catalogue of Life China 2023 Annual Checklist (http://www.sp2000.org.cn/CoLChina) cites 44 species; and Xu et al. (2021) cite 45 species. Of the 57 species of Cynanchum reported by "Flora of China", 26 species now belong to Vincetoxicum (Liu 2023; POWO 2024) (Suppl. material 1) and Cynanchum includes nine species originally housed in allied genera, notably Adelostemma gracillimum (Wall. ex Wight) Hook.f., Graphistemma pictum (Champ. ex Benth.) Maxim., Holostemma ada-kodien Schult., Metaplexis hemsleyana Oliv., M. japonica (Thunb.) Makino, Raphistemma hooperianum (Blume) Decne., R. pulchellum (Roxb.) Wall., Sichuania alterniloba M.G.Gilbert & P.T.Li. (Khanum et al. 2016), and Sarcostemma acidum (Roxb.) Voigt (Liede and Kunze 2002; Liede and Täuber 2002). Besides, three species were missed by the authors of "Flora of China", which are C. defilippii Delponte (Delponte 1871), C. kaschgaricum Y.X.Liou (Liou et al. 1992) and C. lanhsuense T.Yamaz. (Yamazaki 1968). Also, recent studies described three new species of Cynanchum from China (Grubov 2000; Shen et al. 2019; Xu et al. 2021). Currently, 46 species of Cynanchum occur in China.

During a field survey in 2020, we collected an unknown species in Ruili, Yunnan. This species has the typical characters of former Raphistemma species with large and reniform leaves, and large and campanulate corollas. Four names were recorded in Raphistemma: R. ciliata Hook.f. was treated as a synonym of Pergularia daemia (Forssk.) Chiov. (Goyder 2006); R. pulchellum was revised to C. pulchellum (Roxb.) Liede & Khanum (Khanum et al. 2016); R. brevipedunculatum Y.Wan was considered a synonym of R. hooperianum, which has been revised to C. hooperianum (Blume) Liede & Khanum (Li et al. 1995; Khanum et al. 2016). After careful examination of the specimens and literature of former Raphistemma, we are unable to match the newly collected material with any recorded species. Therefore, we here describe the newly collected material as a new species of Cynanchum. Meanwhile, we found significant morphological differences between R. brevipedunculatum and R. hooperianum. We therefore propose to reinstate Raphistemma brevipedunculatum at species level in the genus Cynanchum: since the name is already in use within the genus, a replacement name is necessary and proposed here.

# Material and methods

#### Morphological observation

For the new species, field observations were done and a collection made in Ruili City, Yunnan Province (specimen voucher: Si-Jin Zeng & Lin-Ya Zeng SJ4825 (IBSC)). We collected a living sample of R. brevipedunculatum from the type locality (Longhushan Nature Reserve, Longan County, Guangxi Zhuang Autonomous Region (specimen voucher: Miao Liao LM78 (IBSC)). Literature referring to Cynanchum pulchellum, C. hooperianum, and R. brevipedunculatum was consulted, including protologues (Blume 1826; Wan 1983) and other literature (Wallich 1831; Decaisne 1844; Backer and Bakhuizen van den Brink 1965; Li et al. 1995; Khanum et al. 2016). Specimens of C. hooperianum, C. pulchellum, and R. brevipedunculatum at CANT, GXMI, HIB, IBSC (herbaria acronyms follow Thiers 2022) and specimen images from the Chinese Virtual Herbarium (CVH, http://www.cvh.ac.cn), GXSP, National Specimen Information Infrastructure (NSII, http://www.nsii.org. cn), the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/), the JSTOR Global Plants database (https://plants.jstor.org/), P (http://science. mnhn.fr/institution/mnhn/collection/p/item/search/form), and TI (http://umdb. um.u-tokyo.ac.jp/DShokubu) were checked. Morphological data of R. brevipedunculatum is based on living plants, specimens collected in the field and protologues (Wan 1983). Characteristics of C. hooperianum are taken from Decaisne (1844) and Backer and Bakhuizen van den Brink (1965) and those of C. pulchellum from Wallich (1831) and Backer and Bakhuizen van den Brink (1965).

# **Phylogenetic analysis**

DNA extraction, sequencing, assembly, and annotation

We obtained total genomic DNA of the new species and *R. brevipedunculatum* from fresh leaf material dried in silica gel with the plant genomic DNA kit (DP305, Tiangen, Beijing, China). The samples were sent to Novogene (Tianjin, China) for library preparation (350 bp) for genome skimming sequencing. We used an Illumina HiSeq 2000 to conduct a paired-end sequencing (150 bp), generating 10 Gb raw data for each sample. After quality control of the raw data using fastp 0.19.7 (Chen et al. 2018: parameter settings fastp -g -q 5 -u 50 -n 15 -l 150), we extracted 3 Gb paired reads for plastid and nuclear ribosome assembly using GetOrganelle v.1.7 with the parameters "-t 30 -R 15 -k 21,45,105,115,127 -F embplant\_pt" and "-t 30 -R 7 -k 35,85,115 -F embplant\_nr" respectively (Jin et al. 2020).

A plastid genome of *Apocynum venetum* L., a continuous sequence (18S-ITS1-5.8S-ITS2-26S) of the ribosome genome of *Asclepias albicans* S.Watson, and the nrETS of *Calciphila galgalensis* (Liede) Liede & Meve and *Cynanchum adalinae* (K.Schum.) K.Schum. (GenBank accession numbers: MT313688, JN665082, LN896997, and LN897003) were used as respective references. We employed Geneious Prime 2019 (https://www.geneious.com) to annotate and extract three plastid DNA markers (one spacer of *trnL-F* and two introns of *rps16* and *trnL*) and two nuclear DNA regions (nrITS: internal transcribed spacer; nrETS: external transcribed spacer). New sequences of the five loci were uploaded to GenBank with accession numbers OP810602–OP810613 and OP853101– OP853103 (https://www.ncbi.nlm.nih.gov/).

#### Taxa sampling, alignment, and phylogenetic analysis

Sequences of the new species and *R. brevipedunculatum* were added to a reduced matrix of Khanum et al. (2016) with combined data of five loci (nrETS, nrITS, *rps16*, *trnL*, and *trnL-F*) for phylogenetic analysis (Suppl. material 2). We used 57 samples for molecular analysis, including 50 samples (49 species) of *Cynanchum*, covering all nine clades in Khanum et al. (2016). Of the former *Raphistemma* species, we included *C. pulchellum* and *C. hooperianum* (in fact a specimen belonging to *R. brevipedunculatum*). Seven samples were taken as outgroups: *Pentatropis madagascariensis* Decne., *P. nivalis* (J.F.Gmel.) D.V.Field & J.R.I.Wood, and *P.* sp. which belong to subtribe Tylophorinae, and *Calciphila galgalensis* (including two samples), *Calciphila gillettii* Liede & Meve and *Calotropis procera* (Aiton) W.T.Aiton which belong to subtribe Asclepiadinae.

We aligned sequences of the three plastid markers and two nuclear regions separately using the MUSCLE optional in the software MEGA v.7.0.26 (Kumar et al. 2016) and concatenated the data using the Concatenate Sequences or Alignments optional in Geneious Prime 2019 (https://www.geneious.com). The incomplete sequences were filled with missing data.

We used RAxML-HPC2 8.2.12 on XSEDE (Stamatakis 2014) through the CIP-RES portal (Miller et al. 2010) to perform Maximum likelihood (ML) analysis by using the GTRCAT model and 1 000 bootstraps. Finally, we viewed and edited the tree in Figtree 1.4.2 (Rambaut 2012).

# Results

#### Morphological comparison

The new species resembles *Cynanchum hooperianum*, *C. pulchellum*, and *Raphistemma brevipedunculatum*, but differs from them by the broadly ovate corolla lobes, purple-red corolla, and connivent corona apex slightly exceeding the corolla throat (Table 1).

Raphistemma brevipedunculatum is significantly different from Cynanchum hooperianum by the longer calyx lobes (5–6 mm vs. 3–4 mm), the longer corolla-tubes (12–16 mm vs. 8–9 mm), the oblong corolla lobes (vs. ovate), and the corona lobes with connivent corona-scales apex, not exceeding the throat of

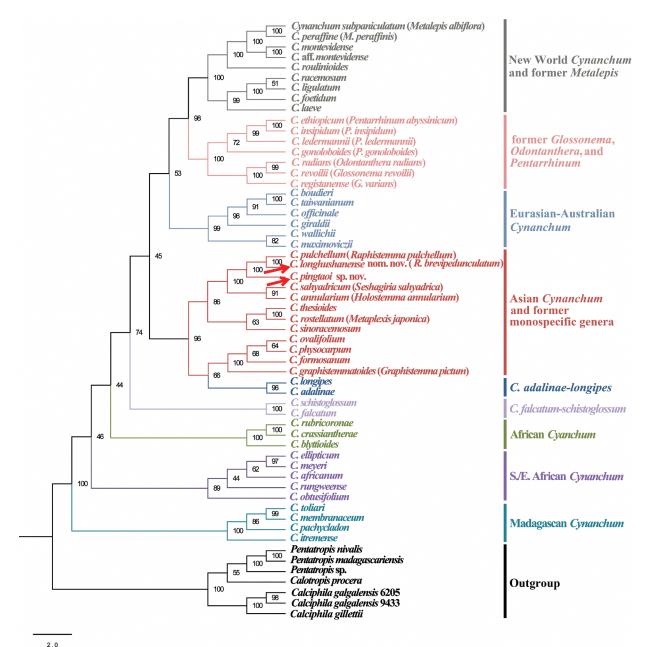
Characters	Cynanchum pingtaoi	Cynanchum hooperianum	Cynanchum pulchellum	Cynanchum longhushanense (Raphistemma brevipedunculatum)
Calyx length	ca. 6 mm	3-4 mm	3-4 mm	5-6 mm
Corolla-tube length	12-14 mm	8–9 mm	12-18 mm	12-16 mm
Corolla lobe shape	Broadly ovate (Fig. 4h)	Ovate	Ovate-oblong	Oblong (Fig. 2i)
Corolla color	Outer surface greenish- white, inner surface purple-red (Fig. 4h)	Outer surface light green, inner surface white, with purple spots near the top of corolla lobe	Outer surface slightly greenish, inner surface white	Outer surface greenish- white, inner surface white (Fig. 2i)
Corona-scales apex	Connivent, corona apex slightly exceeding corolla throat (Fig. 3e)	Separate, reaching the middle of the corolla-segments or further	Barely connivent, slightly exceeding corolla throat	Connivent, not exceeding corolla throat (Fig. 2h)
Follicles	Fusiform, with three ribs	Not observed	Oblong, without ribs	Fusiform, with three ribs

Table 1. Morphological comparison of Cynanchum pingtaoi and closely related species.

the corolla (vs. corona-scales apex separate, reaching the middle of the corolla-segments or further). *Raphistemma brevipedunculatum* flowers in June–July (Wan 1983), but *Cynanchum hooperianum* flowers almost all year (Blume 1826).

# **Molecular phylogenetics**

The combined phylogenetic analysis shows that the new species forms a monophyletic clade (BS = 100%, Fig. 1, Suppl. material 3) with two former *Raphistemma* species. The two former *Raphistemma* species (*C. pulchellum* and *R. brevipedunculatum*) are sister species (BS = 100%, Fig. 1, Suppl. material 3).



**Figure 1.** Simplified maximum likelihood tree of *Cynanchum* based on two nuclear regions (nrETS and nrITS) and three plastid markers (*rps16* and *trnL* introns, and *trnL-F* spacer). Bootstrap support values are given for each node. See Suppl. material 3 for maximum likelihood tree. Clade designations follow those of Khanum et al. (2016) and names in parentheses are the corresponding names that were used in Khanum et al. (2016).

#### **Taxonomic treatments**

*Cynanchum longhushanense* G.D.Tang & Miao Liao, nom. nov., non *Cynanchum brevipedunculatum* J.Y.Shen et al. (2019: 217). urn:lsid:ipni.org:names:77339615-1 Fig. 2

 $\equiv$  Raphistemma brevipedunculatum Y.Wan, in Guihaia 3(3): 197 (1983).

**Type.** CHINA. Guangxi: Longan County, Longhushan Nature Reserve, open woods, 2 Jul. 1981, *D.H. Tan 81329* (holotype: GXSP[GXSP0000038!]; isotype: CANT[CANT00002128!]).

Chinese name. Guangxi Dahuateng (广西大花藤).

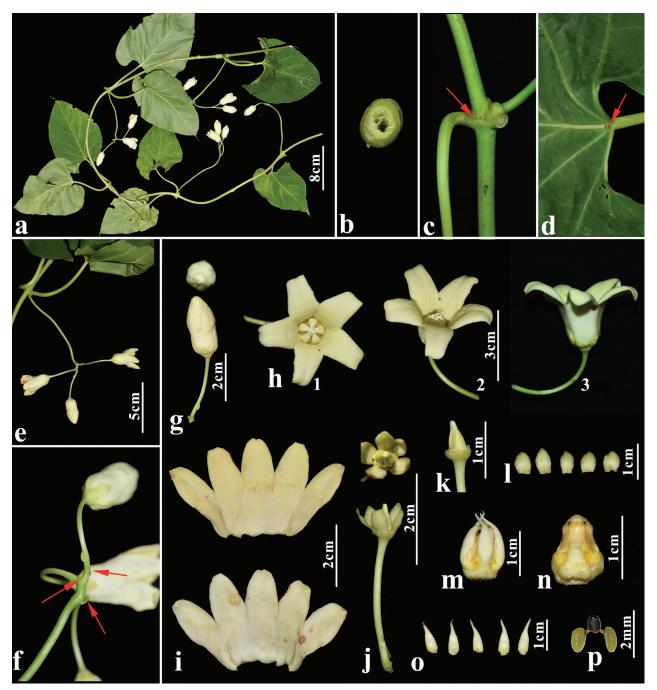
Notes. Raphistemma brevipedunculatum was considered a synonym of R. hooperianum (Li et al. 1995), which was transferred to Cynanchum as C. hooperianum (Khanum et al. 2016). Although C. hooperianum is not included in our molecular study, morphological differences between specimens of C. hooperianum and R. brevipedunculatum are significant. We therefore reinstate R. brevipedunculatum as a distinct species of Cynanchum. We renamed Raphistemma brevipedunculatum as Cynanchum longhushanense because the specific epithet 'brevipedunculatum' is occupied in Cynanchum (C. brevipedunculatum J.Y.Shen). C. longhushanense differs from C. hooperianum by the longer calyx lobes (5-6 mm vs. 3-4 mm), the longer corolla-tubes (12-16 mm vs. 8-9 mm), the oblong corolla lobes (vs. ovate), and the corona lobes with connivent corona-scales apex, not exceeding the throat of the corolla (vs. corona-scales apex separate, reaching the middle of the corolla-segments or further). It should be noticed that in the protologue of Cynanchum longhushanense ( $\equiv R$ . brevipedunculatum) (Wan 1983), the number of glands at the base of the calyx was described as five, but we observed 10-15 glands per calyx in our Guangxi collection (Fig. 2j). More specimens are expected to be collected to determine the morphological variation.

**Other specimens examined.** CHINA. Guangxi: Longan County, Pingshan Village, 10 Oct. 1977, *Longan Investigation Team 2-040* (GXMI[GXMI031735!]); Longan County, Longhushan Nature Reserve, 14 Nov. 1982, *Y. Wan & Rui-Ju Liu 82430* (paratype: GXSP[GXSP0000039!]); Longan County, Longhushan Nature Reserve, 25 Jun. 2021, *Miao Liao LM78* (IBSC).

*Cynanchum pingtaoi* S.Jin Zeng, G.D.Tang & Miao Liao, sp. nov. urn:lsid:ipni.org:names:77339616-1 Figs 3, 4

**Type.** CHINA. Yunnan: Dehong Dai and Jingpo Autonomous Prefecture, Ruili City, Nongdao Town, Tongbiguan Provincial Nature Reserve, 23°57'N, 97°32'E, elev. 839 m, 24 Aug. 2020, *Si-Jin Zeng & Lin-Ya Zeng SJ4825* (holotype, IBSC[IBSC1009908!]; isotypes, IBSC [IBSC1009907!], [IBSC1009909!], [IBSC1009910!]).

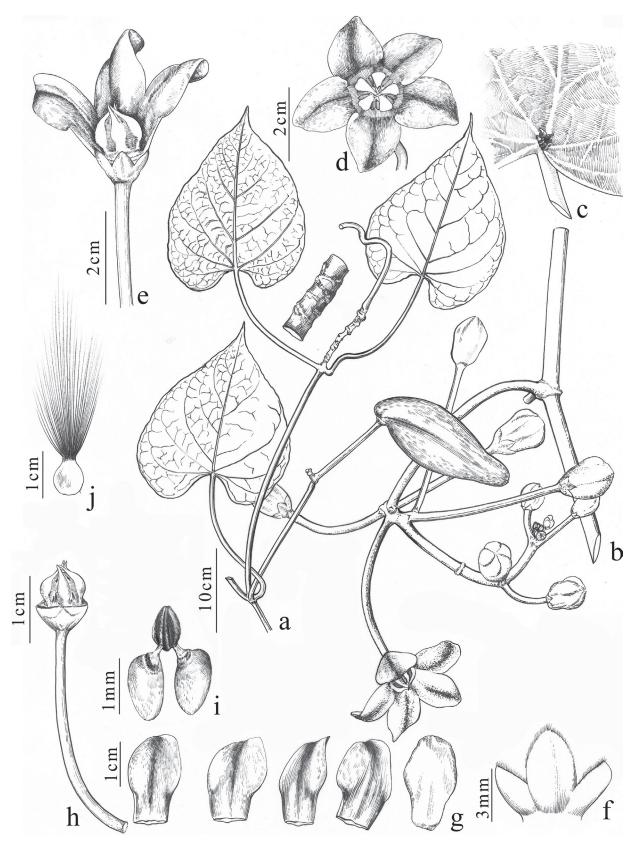
**Diagnosis.** Cynanchum pingtaoi resembles C. longhushanense, differing by its broadly ovate corolla lobes (vs. oblong), the purple-red inner surface of the



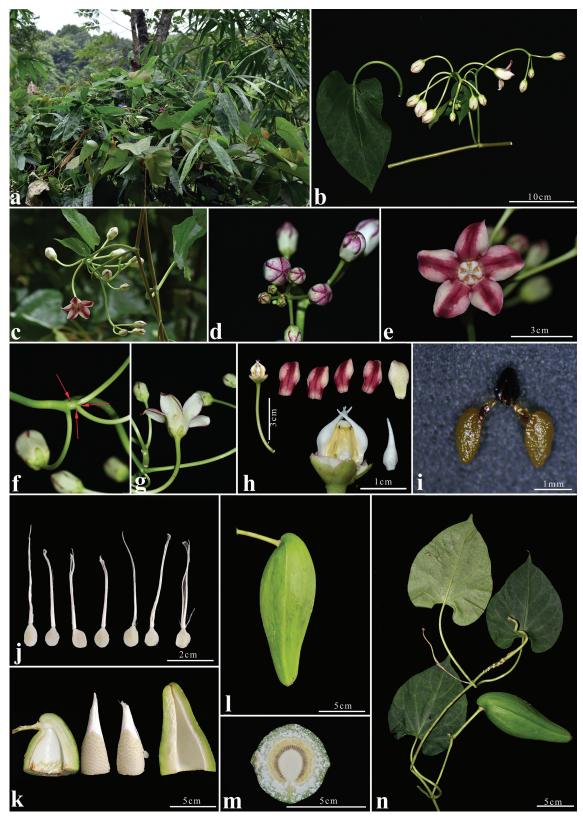
**Figure 2.** *Cynanchum longhushanense* G.D.Tang & Miao Liao **a** flowering branch **b** cross-section of hollow stem **c** node with small glands **d** petiole with glands at the top **e** inflorescence **f** bracteoles at the base of the pedicel **g** flower bud (above: top view; below: side view) **h** flower (1) top view, (2) lateral view, showing the connivent corona-scales apex, not exceeding the throat of the corolla, (3) side view, showing corolla lobes overlapping to the right and corolla tube **i** opened corolla, adaxial (above) and abaxial (below) view **j** ovary with calyx (above: top view, showing glands at the base of the calyx, below: side view of ovary, calyx and pedicel) **k** ovary **l** calyx lobes **m** gynostegium with corona lobes **n** gynostegium **o** corona lobes **p** pollinarium. All photos based on *Miao Liao LM78*.

corolla (vs. white), and the corona-scales apex connivent, slightly exceeding the corolla throat (vs. corona-scales apex connivent, not exceeding the throat of the corolla).

**Description.** Twining liana. White latex in stems and leaves. **Branchlets** fistulous, smooth, glabrous, slightly woody. **Leaves** opposite; petiole 6–14



**Figure 3.** *Cynanchum pingtaoi* S.Jin Zeng, G.D.Tang & Miao Liao **a** follicle and leaves **b** inflorescence **c** glands at the base of leaf **d** top view of flower **e** side view of flower with two corolla lobes removed **f** calyx **g** corolla lobes (each lobe attached to part of the corolla tube), outer surface on the right, inner surface on the left (four drawn) **h** corolla separation, showing gynostegium with corona lobes **i** pollinarium **j** seed. Illustration based on *Si-Jin Zeng & Lin-Ya Zeng SJ4825* (IBSC), and drawn by Ding-Han Cui.



**Figure 4.** *Cynanchum pingtaoi* S.Jin Zeng, G.D.Tang & Miao Liao **a** habitat **b** inflorescence and leaf **c** inflorescence **d** flower buds **e** top view of flower **f** bracteoles at base of pedicel **g** side view of flower **h** corolla and corona separation, showing gynostegium, corona and corolla lobes (outer surface: upper right hand corner; inner surface: to the left of the outer surface) **i** pollinarium from dry specimen **j** young seeds **k** longitudinally opened half follicle showing seed arrangement I follicle, different views to show the three ribs **m** transverse section through follicle **n** follicle and leaves. All photos based on *Si-Jin Zeng & Lin-Ya Zeng SJ4825* (IBSC).

cm long, smooth, sparsely white puberulent, later glabrescent, with small yellowish-brown glands at the apex, nodes with small glands; leaf blade deeply cordate to reniform, 7-15 × 4-13 cm, membranous, base cordate, apex acuminate, margin entire, adaxial surface dark green, glabrous, abaxial surface light green, sparsely white puberulent on veins, gradually glabrescent later; basal veins five or seven, palmate, secondary veins three to five pairs, pinnate, tertiary veins reticulate, smooth adaxially, raised abaxially. Inflorescences extra-axillary, subumbellate to subracemic, 5-11 flowers; peduncle 10-12 cm long, smooth; pedicel 3.0-5.5 cm long, smooth, sparsely white puberulent near the base, base with bracteoles triangular, ca.  $0.1 \times 0.1$  cm. Calyx yellowish green, basally fused, lobes elliptic, ca. 0.6 × 0.5 cm, inside the base with small glands, apices obtuse, margins ciliate. Corolla campanulate, glabrous, external surface greenish white, inner surface purple-red, 3.3-3.5 cm in diam; lobes slightly longer than tube, tube 1.2-1.4 cm, lobes broadly ovate, 1.3-1.8 × 1-1.2 cm, apices reflexed, overlapping to the right. Corona lobes linear-subulate, white, separate, ca. 1.1 cm long, inserted at base of gynostegium, longer than gynostegium, corona-scales apex connivent, slightly exceeding corolla throat. Anthers ca. 0.7 × 0.4 cm, apices with inwardly incurved wings. Stigma broadly rounded, slightly depressed, white. Pollinia 2 per pollinarium, ellipsoid, yellow, pendulous, ca. 0.13 × 0.08 cm, caudicle ca. 0.05 cm long, retinaculum ca. 0.1 cm long. Follicles solitary, fusiform, ca. 14.5 cm long, ca. 5 cm diam., glabrous, with a thick fibrous pericarp, triangulate, apex curved outwards; seeds ovoid, 0.8 cm × 0.6 cm, tipped with a white silky coma; coma 3.8-4.2 cm long.

**Etymology.** The specific epithet *pingtaoi* honors the eminent botanist Ping-Tao Li (李秉滔), who is an expert in the Apocynaceae.

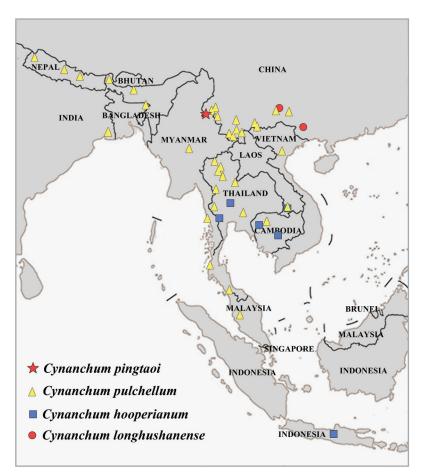
Chinese name. Bingtao Dahuateng (秉滔大花藤).

**Distribution.** Endemic to China. Only one population was found at the border of China-Myanmar in Ruili, Yunnan Province, China. Fig. 5.

Habitat and phenology. This species occurs near open woods at an elevation of about 850 m. Flowering was observed from September to October, fruiting from November to December.

**Provisional IUCN assessment.** The species is currently known only from the type locality, where only a few individuals were seen. Suitable habitat exists in the proximity of the type locality. Nevertheless, as there is no reliable information on the population size or distribution of this species, we propose to treat it as Data Deficient (IUCN 2019).

**Notes.** The large and reniform leaves, campanulate corolla, and lanceolate corona indicate that this species is morphologically close to the former *Raphistemma* species, which have been included in *Cynanchum* (Khanum et al. 2016). The molecular phylogenetic analyses also showed that *Cynanchum ping-taoi* is sister to *C. pulchellum* + *C. longhushanense* (Fig. 1; Suppl. material 3). We do not currently possess molecular data of *C. hooperianum*, but the new species can be distinguished from this species by the purple-red inner surface of the corolla (vs. white inner surface of the corolla with purple spots near the top of the corolla lobes), the connivent corona-scales apex, slightly exceeding the corolla-segments or somewhat further), the longer calyx-segments (6 mm vs. 3–4 mm), and the longer corolla-tubes (12–14 mm vs. 8–9 mm).



**Figure 5.** Distribution map of *Cynanchum pingtaoi* and the three *Cynanchum* species formerly were considered to belong to *Raphistemma* based on specimens cited in Suppl. material 4. The base map [No. GS(2016)1666] was downloaded from http://bzdt.ch.mnr.gov.cn/.

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# **Additional information**

# **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Miao Liao: Data curation (Lead); Formal analysis (Lead); Investigation (Supporting); Methodology (Lead); Software (Lead); Validation (Equal); Visualization (Lead); Writing – original draft (Lead); Writing – review & editing (Equal). Si-Jin Zeng: Conceptualization (Supporting); Formal analysis (Equal); Investigation (Lead); Writing – review & editing (Supporting). Lin-Ya Zeng: Investigation (Supporting); Resources (Supporting). Hai-Jun Yin: Investigation (Equal). Mao-Lin Yan: Investigation (Equal). Cai-Fei Zhang: Data curation (Equal); Validation (Equal); Writing – review & editing (Supporting). Guang-Da Tang: Conceptualization (Lead); Data curation (Equal); Formal analysis (Supporting); Funding acquisition (Lead); Methodology (Supporting); Project administration (Lead); Resources (Supporting); Supervision (Lead); Validation (Lead); Writing – review & editing (Lead).

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#### Data availability

The newly obtained sequences of *Cynanchum pingtaoi* and *C. longhushanense* have been submitted to the NCBI website. The DNA sequence data supporting the findings of this study are available in Suppl. material 2.

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# **Supplementary material 1**

#### List of species names changes within Cynanchum in Flora of China

- Authors: Miao Liao, Si-Jin Zeng, Lin-Ya Zeng, Hai-Jun Yin, Mao-Lin Yan, Cai-Fei Zhang, Guang-Da Tang
- Data type: xlsx
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Link: https://doi.org/10.3897/phytokeys.241.111499.suppl1

# Supplementary material 2

#### List of ingroup and outgroup taxa used in the phylogenetic analyses

Authors: Miao Liao, Si-Jin Zeng, Lin-Ya Zeng, Hai-Jun Yin, Mao-Lin Yan, Cai-Fei Zhang, Guang-Da Tang

Data type: xlsx

- Explanation note: List of ingroup and outgroup taxa used in the phylogenetic analyses with voucher information (geographic origin, collection, herbarium) and Genbank accession numbers. "\*" indicates new sequences; "-" indicates missing sequences; bold GenBank accession numbers indicate connected sequences for *trnL* intron and *trnL-F* intergenic spacer.
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# **Supplementary material 3**

#### Maximum likelihood tree of Cynanchum

Authors: Miao Liao, Si-Jin Zeng, Lin-Ya Zeng, Hai-Jun Yin, Mao-Lin Yan, Cai-Fei Zhang, Guang-Da Tang

Data type: pdf

- Explanation note: Maximum likelihood tree of *Cynanchum* based on two nuclear regions (nrETS and nrITS) and three plastid markers (*rps16* and *trnL* introns, and *trnL-F* spacer). Bootstrap support values are given for each node.
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# Supplementary material 4

# Specimens of Cynanchum hooperianum, C. longhushanense, C. pingtaoi, and C. pulchellum used for the distribution map

Authors: Miao Liao, Si-Jin Zeng, Lin-Ya Zeng, Hai-Jun Yin, Mao-Lin Yan, Cai-Fei Zhang, Guang-Da Tang

Data type: docx

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